IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Klinman et al.
Application No. 10/682,130
Filed: October 7, 2003
Confirmation No. 1348
For: METHOD OF TREATING INFLAMMATORY LUNG DISEASE WITH SUPPRESSORS OF CPG OLIGONUCLEOTIDES
Examiner: Nina Archie
Art Unit: 1645
Attorney Reference No. 4239-66902-01

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DECLARATION UNDER 37 C.F.R § 1.132

1. I, Ihsan Gursel, am currently an associate professor at Bikent University in Ankara Turkey. I received by PhD. from the Department of Biological Sciences, Middle East Technical University. I also have worked as a Staff Scientist at the Food and Drug Administration, the Center for Biologics Evaluation and Research in the United States. My work focuses on the immunomodulatory properties of bacterial and mammalian DNA. I have designed controlled release systems for oligodeoxynucleotide (ODN) targeting and delivery, and I am an expert in the determination of gene expression and transcriptional profiling of immune cells, and the evaluation of immunoprotective, anti-cancer and immunoadjuvant effects of synthetic ODNs.

It is my understanding that claims 35, 46, 47, 54 and 60-74, 76-78 and 80-87 of the above-referenced patent application are rejected under 35 U.S.C. 112, first paragraph, as allegedly not being enabled by the specification.

2. The pending claims of the above-referenced patent application are limited to methods of reducing TNFα, MIP-2, IL-6 and KC expression using suppressive ODNs that comprise the sequences of SEQ ID NO: 1 (claim 35), SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 21, or a combination of two or more thereof (claim 46 and 54). These

ODNs are at most 30 nucleotides in length, and have two to four guanosine rich sequences and form a G-tetrad. Claims 60, 70-74 and 78 are limited to methods for reducing the expression of TNFα, MIP-2, IL-6 and KC wherein the ODN consists of SEQ ID NO: 1, SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 21.

3. Using the guidance provided by the specification, I synthesized ODNs having the nucleic acid sequences set forth as SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19. My lab tested the effect of these ODNs on the secretion of TNF-α and IL-6. My lab also tested the effects of these ODNs on the secretion of MIP-1a and MIP-3a. The assays used in these studies were similar to those disclosed in the specification, see for example page 25.

As disclosed in the specification, CpG ODN stimulates cells, such as RAW 246.7 cells or primary spleen cells to produce cytokines. Thus, two K-type CpG ODNs, K3 and K23, were used to stimulate mouse spleen cells to produce TNF-α, IL-6, MIP-1a and MIP-3a. Adding equimolar concentrations of ODNs having the nucleic acid sequences set forth as SEQ ID NO: 14 (A17), SEQ ID NO: 17 (A21), SEQ ID NO: 18 (A22) and SEQ ID NO: 19 (H154) and SEQ ID NO: 21 (15027DG) suppressed cytokine secretion, as shown in the table below:

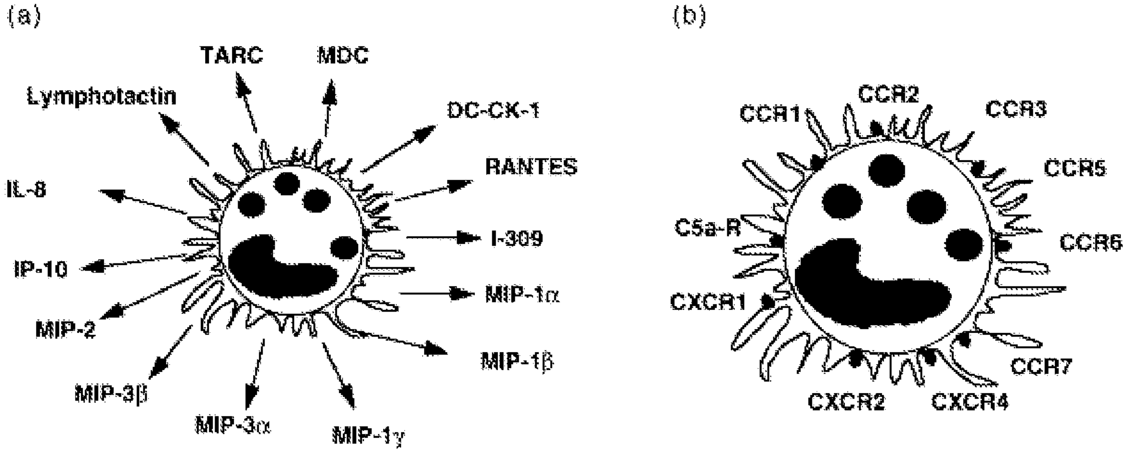
	% Suppression of Cytokine Secretion from K23 (or K3) plus Supp							
SEQUENCES	**TNFa**	SD	**IL6**	SD	**MIP3a**	SD	**MIP1a**	SD
(A17) TGGGCGGTTCAAGCTTGA	**81.1**	3.2	**88.4**	7.6	**81.9**	3.3	**79.7**	4.8
(A21) TCAAGCTTCAGGGGGG	**84.2**	6.1	**83.9**	3.3	**74.5**	5.4	**80.2**	4.2
(A22) GGGGGGTCAAGCTTCA	**71.2**	4.4	**88.5**	4.8	**78.9**	6.9	**81.1**	7.3
(H154) CCTCAAGCTTGAGGGG	**84.5**	6.3	**89.6**	9.5	**79.9**	12.3	**80.8**	5.5
(15027DG) GAGCAAGCTG*G*ACCTTCCAT	**14.4**	3.4	**19.3**	4.4	**ND**	ND	**ND**	ND

ND = not done

The numbers presented in the table are based on the ratio of stimulation with CpG ODN sequences to CpG ODN plus treatment with the indicated suppressive ODN. Each experiment was performed in triplicate wells, and the experiment was repeated at least three independent times. The numbers presented (the percent suppression level) were based on baseline cytokine levels of TNFa [ng/ml]: 129.6 ± 9.1; IL6 [ng/ml]: 159.7 ± 13.9; MIP3a [ng/ml]: 45.5 ± 6.9; MIP1a [ng/ml]: 89.0 ± 12.3. The results are statistically significant.

Thus, ODNs were tested having the nucleic acid sequences set forth as SEQ ID NO: 14 (A17), SEQ ID NO: 17 (A21), SEQ ID NO: 18 (A22), and SEQ ID NO: 19 (H154) greatly suppressed cytokine secretion using the methods provided in the specification. All of these ODNs significantly reduced the expression of TNF-α, IL-6, MIP-3a and MIP-1a.

4. We did not assess the effect of these ODNs on the production of MIP-2. However, the effect of these ODNs on MIP-2 should be equivalent to these effects. MIP-1a, MIP-2 and MIP-3a are all produced by dendritic cells (see Rescigno et al., Immunology and Cell Biology 77: 404-410, 1999, figure 4, which is reproduced below).



Thus, MIP-2 production should be similarly suppressed.

5. The expression of keratinocyte derived cytokine (KC) also was not measured in these experiments. However, we expect KC concentration to be decreased by SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 21, to a similar extent. Specifically, since MIP and KC are in the same chemokine cascade (see Luo et al., J. Immunology 165: 4015-4023, 2002), KC production would be suppressed similarly.

6. All statements made herein and of my own knowledge are true and all statements made on information are believed to be true; and further, these statements were made with the knowledge that willful false statements and like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date 16 June 2010

Ihsan Gursel, Ph.D.